

Suppression of Alveolar Macrophage Membrane Receptor-Mediated Phagocytosis by Model and Actual Particle-Adsorbate Complexes. Initial Contact with the Alveolar Macrophage Membrane

by George J. Jakab,* Terence H. Risby,* Shelley S. Sehnert,* Robert R. Hmielecki,* and Jane E. Farrington†

Alveolar macrophages were treated with carbon blacks and adsorbates in order to evaluate the biologic effect of adsorbate, adsorbent and adsorbate-adsorbent complexes. Their capacity to phagocytize a subsequent challenge via the Fc-membrane receptor was quantified. Phagocytosis was suppressed in a dose-related manner with increasing concentrations of both carbon blacks and adsorbates. Carbon black N339 covered with 0.5 monolayers of the adsorbates suppressed phagocytosis more than N339 without the adsorbates. Increasing the adsorbate acrolein coverage from 0.5 to > 2.0 monolayers suppressed phagocytosis in a dose-related manner. Finally, samples of diesel particulate matter collected from an engine operated on a pure hydrocarbon fuel with various oxidizers, air (PSU #1) and an oxidizer free of nitrogen (N-free) were tested. Treatment of the macrophages with PSU #1 had a negligible effect on phagocytosis whereas the N-free sample suppressed phagocytosis in a dose-related manner. The data show that alveolar macrophage Fc-receptor-mediated phagocytosis is affected by: carbon black and adsorbate identity and concentration, coverage of the carbon black with adsorbates, and the oxidizer used in the generation of particles emitted by a diesel engine.

Introduction

The relationship between the physical chemistry of particle-pollutant interactions and inhalation toxicology is the focus of this on-going research program. Pollutant gases or vapors sorbed on the surfaces of inhalable environmental particles may reach the distal regions of the respiratory tract that are normally inaccessible to such pollutants. In combination, the particle and the adsorbed gas may produce physiological effects not induced by either agent alone (1,2).

The first line of defense against inhaled particles in the distal lung is the alveolar macrophage phagocytic system (3). Particles reaching the alveolar regions are rapidly ingested by the macrophages thereby effectively sequestering the deposited material from the vulnerable respiratory surface. Phagocytic uptake is initiated by the attachment of the particles to macrophage membrane receptors. The Fc receptor recognizes the Fc portion of immunoglobulin (IgG) and facilitates the ingestion of opsonized particles. Since one of the first sites of contact of inhaled particles is the macrophage membrane, the effect of *in vitro* treatment of alveolar macrophages with carbon, adsorbate, and carbon-adsorbate combinations on the phagocytic activity of macrophages was tested. The response of the macrophages to a secondary particle challenge is dependent on Fc receptor-mediated phagocytic ingestion.

Carbon black alone is not an environmental hazard and is considered a nuisance dust (4-6) since upon inhalation, the particles do not appear to produce profound toxic

*Division of Environmental Chemistry and Biology, Department of Environmental Health Sciences, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, MD 21205.

†The Oncology Center, The Johns Hopkins University, School of Medicine, Baltimore, MD 21205.

Address reprint requests to G. J. Jakab, Department of Environmental Health Sciences, Johns Hopkins School of Hygiene and Public Health, 615 N. Wolfe Street, Baltimore, MD 21205.

effects in the lung parenchyma. Pollutant molecules sorbed on the surface of carbon black may alter the biologic response to the inhaled carbon black if the sorbed molecules are released from the surface of the particle. The carbon blacks (adsorbents) selected for testing have different surface areas and degrees of surface oxidation. They are used in this study as models for more complex environmental particles. The model pollutant molecules (adsorbates) used contain many of the functional groups common to the adsorbed molecules found on particles emitted during combustion of organic materials. The rationale for this study was to test the biologic effect of simple particle-adsorbate complexes that model many of the important physical and chemical parameters of more complex particulate-adsorbate combinations, in order to predict the biologic effects of actual environmental pollutants emitted during the incomplete combustion of organic material.

After deposition in the distal lungs, the adsorbed molecules may be released from the particle surface into lung tissue or fluid. Release may occur by either physical or chemical (metabolic) processes to provide a focused dose to the lung epithelium.

The intermolecular parameters that describe the identity and amount of gas phase molecules adsorbed on the surface of model environmental particles have been quantified as a function of the physicochemical properties of model adsorbents and adsorbates (7). The conclusions were that the major factors that determine the strength of adsorption are the surface properties of the adsorbent, the intermolecular forces between the surface, and the adsorbing molecule and the surface coverage of the adsorbent. The study was subsequently continued by modeling the physical release of these adsorbed molecules into pure solvents, liposomes, or zwitterionic micelles that model the surfactant-rich physiological fluid found in the alveolar region of the lung (8,9). It was concluded that the major factors determining release were the surface properties of the adsorbent, the intermolecular forces between the surface and the adsorbing molecule, the surface coverage of the adsorbent, and the polarity of the physiological fluid interacting with the adsorbate-adsorbent complex.

Phagocytic cells such as alveolar macrophages could provide an alternate means of release of adsorbed molecules. There is evidence that exogenous molecules can be metabolized within phagocytic cells to more polar metabolites, thus facilitating detoxification and subsequent excretion (10). If a cell containing such hydrophilic metabolites is lysed, the cellular contents would not be predisposed to re-adsorption onto any nonpolar particle surface. Rephagocytosis of metabolites would be less likely, although they could be ingested via pinocytosis. Polar metabolites are likely to remain in solution in the alveolar surfactant, and would have increased probability for interaction with lung epithelium. Nonpolar molecules that are metabolized more slowly could be cycling between alveolar surfactant and resident nonpolar particles and may remain in the deep lung for longer periods

of time. Residence time will be further enhanced if the nonpolar particle does not elicit a significant inflammatory response leading to an increased influx of phagocytic cells to the lung. Additionally, postphagocytic events may lead to macrophage lysis and release of any unmetabolized nonpolar molecules onto the lung epithelium, which is the deposition and residence site of the nonpolar particle prior to clearance.

On the basis of this discussion, it may be postulated that adsorbed molecules that are not readily released into alveolar surfactant by physical processes may be released by metabolic processes involving intracellular enzymes. Intracellular release requires phagocytosis. The process itself begins with the interactions of receptors on the cell exterior membrane with the particle-adsorbate complex. This paper describes the alterations of the reserve capacities of alveolar macrophages to phagocytize a subsequent challenge after prior exposure to adsorbates, adsorbents or adsorbent-adsorbate complexes. Phagocytosis begins with the contact of the alveolar macrophage surface by the particle adsorbate complex.

Materials and Methods

Chemicals

The adsorbates studied were acetophenone, acrolein, benzofuran, nitrobenzene and thiophene (>99 + % purity, Aldrich or Burdick & Jackson Chemicals). The adsorbents used were oil furnace carbon blacks and their ASTM classifications are as follows: N765, N339, N110, N339 oxidized (N339ox), Black Pearls 2000 (BP 2000) (Cabot Corporation).

Sample Preparation

All samples were prepared in tissue culture medium 199 (TCM 199). The adsorbates were assayed at concentrations of 10, 1.0, 0.1, and 0.01 $\mu\text{g/mL}$. The adsorbents tested were untreated and solvent extracted carbon blacks. The concentrations used were 1.0, 0.1, 0.04, and 0.01 $\mu\text{g/mL}$. All samples with particles were sonicated for 30 min before addition to the macrophage monolayers in order to uniformly disperse the particles within the media and eliminate agglomerate formation.

For removal of endogenous pyrolytic products from the surfaces of the carbon blacks, the blacks were packed in chromatographic columns and a mixture of equal parts of 1-hexane, dichloromethane, and methanol (Aldrich, >99 + % pure) were eluted through the columns using an HPLC system (Varian 2000 series), as previously described (8).

Exogenous adsorbates were sorbed on the carbon blacks by previously described methods (8). The procedure consisted of injecting a known amount of adsorbate in the gas phase via a heated injection port of a gas chromatograph (Varian 3700 series) onto a column packed with a known weight of carbon black which was maintained at ambient temperature. Desorption of the

adsorbates does not occur at ambient temperatures (7). This was experimentally confirmed by monitoring column effluent with a flame ionization detector. Classical calculations of the amount of adsorbate on the carbon blacks were employed (7).

Preparation and Collection of Diesel Particles

The diesel particulate matter was obtained by direct sampling of undiluted exhaust gases from the exhaust stack of an AVCO-LYCOMING BERNARD Model W-51 industrial diesel engine. Samples were collected isokinetically on Teflon-coated glass fiber filters and the entire sampling system was maintained at 52°C (11,12). The engine was operated at constant speed and load (2400 rpm, ¾ rack) using a pure hydrocarbon fuel (1:1 volume ratio of 1-tetradecane and 2,2,4-trimethylpentane) which is a model fuel with similar ignition properties to a full-boiling range diesel fuel. Two different oxidizers were used to support the combustion of the diesel engine, air (PSU #1) and a mixture of carbon dioxide (56%), argon (23%), and oxygen (21%) (N-Free). The diesel particulate matter was carefully scraped from the filter and dispersed in TCM 199 at concentrations of 0.004, 0.01, 0.02, 0.04, and 0.1 mg/mL.

Alveolar Macrophage Fc-Membrane Receptor Phagocytic Assay

Alveolar macrophages were obtained and the phagocytic assay performed with modifications of previous described methods (13). Briefly, the lungs of 150 to 200 gm Wistar rats (Hilltop Laboratory Animals, Scottsdale, PA) were lavaged and the cells suspended in TCM 199 at a concentration of 5×10^5 cells/mL. A 0.2-mL aliquot of this suspension was then placed on 22-mm² coverslips in 35 × 10 mm plastic petri dishes and incubated at 37°C for 45 min. After monolayering, the fluid was removed and immediately replaced with 1.5 mL of various concentrations of adsorbent, adsorbate, adsorbate-adsorbent or Diesel particulate matter mixtures prepared in TCM 199. Following 1 hr of incubation at 37°C, the mixtures were removed, the monolayers washed three times with warmed TCM 199, and cell viability was determined by exclusion of trypan blue. Thereafter, Fc membrane receptor-mediated phagocytosis was assayed by adding 1.5 mL of 0.5% suspension of sensitized sheep erythrocytes (RBCs) in TCM 199 and incubating them at 37°C for 45 min. Noningested RBCs were hypotonically lysed by adding distilled water to the monolayers for 10 sec followed by several rinses with TCM 199. The monolayers were then dried, fixed with methanol, and stained with Wright-Giemsa. The stained cell monolayers were read microscopically at 1000× to quantify the percentage of macrophages containing ingested RBCs. In addition, the number of RBCs ingested per actively phagocytic macrophage was also determined. The phagocytic index (total number of RBCs ingested by 100 macro-

phages) was calculated by multiplying the percent of macrophages phagocytic by the mean number of RBCs ingested per phagocytic macrophage. Two hundred macrophages were counted on each monolayer. For each carbon and adsorbate and carbon-adsorbate combination, two to three separate experiments were performed with two to four replicates for each concentration within an experiment. The phagocytic index for the experimental groups was normalized to the daily control values and is presented as a percentage of control.

Electron Microscopy

The monolayered macrophages were washed briefly in media without protein and then fixed with 2% glutaraldehyde, 1% paraformaldehyde in 0.1 M phosphate buffer (PBS, pH 7.4) starting at room temperature, and then placed on ice for 1 hr. After three 10-min washes with cold PBS with 1% sucrose, the cells were post-fixed for 1 hr in 1% OsO₄ made in PBS (without sucrose). Before dehydration, the attached cells were washed three times for 15 min each. Dehydration was through 100% ethanol; the propylene oxide steps were omitted; embedment was in Poly 812 (Polysciences, Inc.). The flat-faced blocks were popped out of the culture dishes and any remaining glass was etched away with hydrofluoric acid. Thin sections (60 nm) were cut on an LKB-V ultramicrotome with a Diatome diamond knife and mounted on 200 mesh copper grids. After staining in uranyl acetate and lead citrate to enhance contrast, the sections were viewed in a Philips 410 transmission electron microscope at 100 KV.

Statistical Analysis

Comparisons of the raw data between controls and treated groups were performed by the Student's *t*-test. All statements of significance are $p < 0.05$.

Results

It was critical that macrophage viability not be strongly affected by the samples assayed. Therefore, the viability of the cells was determined after the initial monolayering (> 95%) and after treatment with the samples (> 90%). The only time that viability was less than 90% was after treatment with the most concentrated samples.

During the course of these studies, approximately 94% of the untreated control macrophages were phagocytic with each phagocytic macrophage ingesting approximately 5.3 particles. Treatment with the adsorbates, carbon or the carbon-adsorbate combinations reduced both the percentage of phagocytic macrophages and the number of particles ingested, thereby lowering the phagocytic index (particles ingested per 100 macrophages).

The effects of adsorbates alone on alveolar macrophage membrane Fc receptor-mediated phagocytic function are presented in Figure 1. Using the phagocytic index as a cumulative measure of phagocytic function,

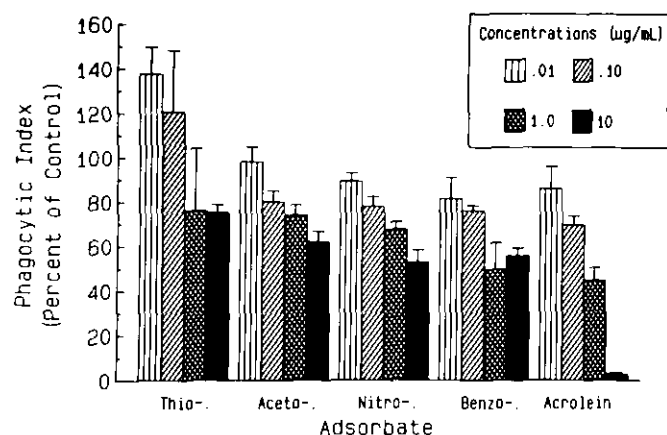


FIGURE 1. Comparison of alveolar macrophage Fc receptor-mediated phagocytosis following 1-hr treatment with increasing concentration of adsorbates. (Thio- = thiophene; aceto- = acetophenone; nitro- = nitrobenzene; benzo- = benzofuran). Each value represents the mean \pm SE of six determinations.

treatment with acetophenone, nitrobenzene, benzofuran, and acrolein suppressed phagocytic activity in a dose-related manner; the most dramatic effect was produced by acrolein. Treatment of the macrophages with thiophene, however, suppressed phagocytosis at the two highest concentrations and appeared to enhance phagocytic activity at the two lowest concentrations. The enhancement however, was not statistically significant ($p > 0.05$).

The effect of carbon black concentration on macrophage Fc receptor-mediated phagocytic function is presented in Figure 2. The carbon blacks tested induced a dose-related suppression of phagocytosis with increasing concentrations. When the carbon black concentration was sufficiently dilute (< 0.1 mg/mL), no aggregation of particles was observed. However, at concen-

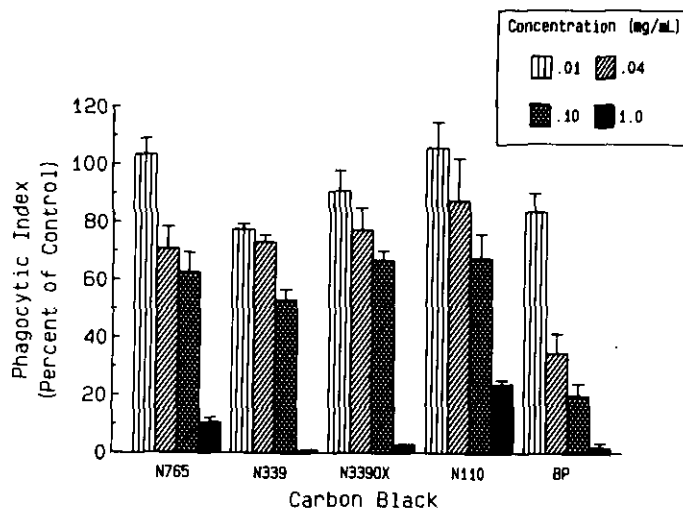


FIGURE 2. Comparison of alveolar macrophage Fc receptor-mediated phagocytosis following 1-hr treatment with increasing concentrations of carbon blacks. Each value represents the mean \pm SE of four determinations.

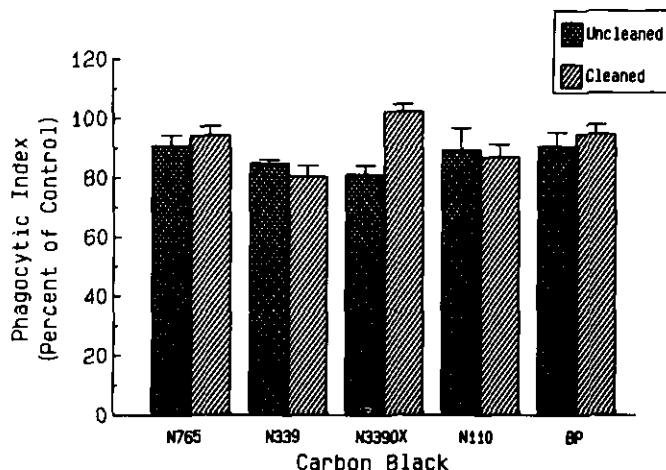


FIGURE 3. Comparison of alveolar macrophage Fc receptor-mediated phagocytosis following 1-hr treatment with native (uncleaned) and endogenous adsorbate extracted (cleaned) carbon black. Each value represents the mean \pm SE of four determinations.

trations of 0.1 mg/mL or greater, the particles agglomerated upon standing and required sonification for dispersion. Since carbon black could be expected to agglomerate during treatment of the macrophages, the dose-related suppression of phagocytosis might be due to either concentration or agglomerate size or both.

The carbon blacks tested are known to have endogenous polycyclic aromatic hydrocarbons sorbed on their surface (14). Because of this, the carbons were compared in their original ("uncleaned") state and after dynamic elution of endogenous adsorbates ("cleaned"). Figure 3 presents the data for the carbon blacks tested at a concentration of 0.04 mg/mL. When the endogenous adsorbates were removed, the phagocytic response did not markedly change. This observation held true for comparison of all the concentrations tested.

To determine the effect of exogenous adsorbates on Fc-receptor-mediated phagocytosis, uncleaned N339 was treated with the adsorbates at a concentration of 0.5 monolayer and then tested at a carbon black concentration of 0.04 mg/mL. The carbon black N339 is representative of nonoxidized carbon blacks. N339 was tested in its uncleaned state because of the likelihood that the results would correlate better with other data generated with uncleaned blacks (7-9,14) and because the data from the former experiment indicated that cleaning did not change the phagocytic response. The carbon black was tested at 0.04 mg/mL because range finding studies had shown that at this concentration sufficient adsorbate was present on the surface to produce phagocytic dysfunction. Figure 4 shows that phagocytic dysfunction was produced by all the carbon black-adsorbate combinations, thereby demonstrating that the presence of exogenous adsorbate can alter the biologic effect of carbon blacks.

To determine whether or not phagocytic dysfunction induced by the carbon black-adsorbate combinations

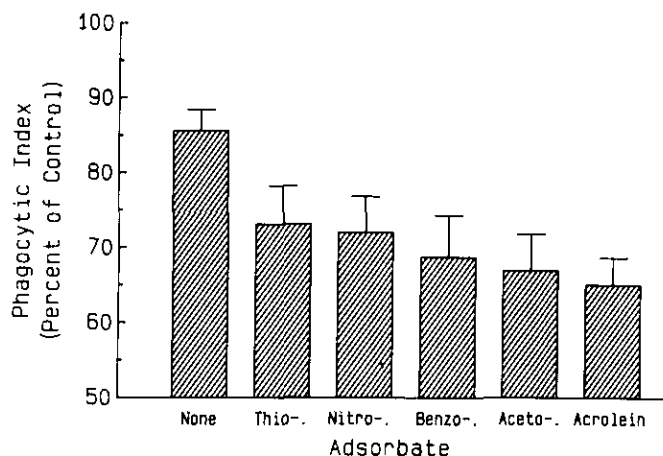


FIGURE 4. Comparison of alveolar macrophage Fc receptor-mediated phagocytosis following 1-hr treatment with 0.04 mg/mL of carbon black N339 at an adsorbate coverage of 0.5 monolayers. (Thio- = thiophene; nitro- = nitrobenzene; benzo- = benzofuran; aceto- = acetophenone). Each value represents the mean \pm SE of four determinations.

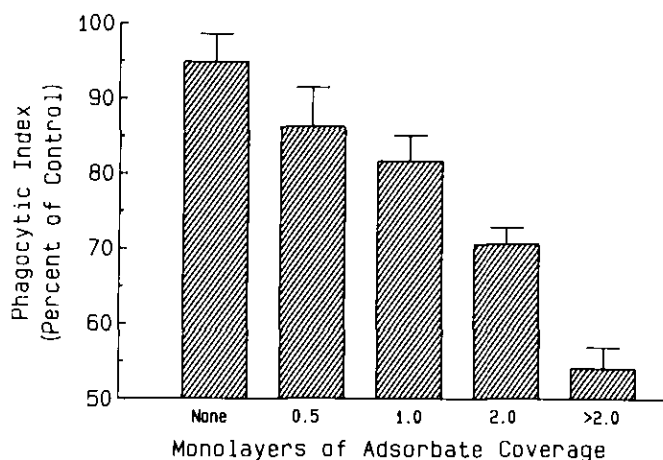


FIGURE 5. Comparison of alveolar macrophage Fc receptor-mediated phagocytosis following 1-hr treatment with 0.04 mg/mL of carbon black N339 at increasing acrolein adsorbate coverages. Each value represents the mean \pm SE of eight determinations.

was due to the physical release of the adsorbates into the tissue culture medium, the carbon black-adsorbate combinations were sonicated for 4 hr in TCM 199. Thereafter, the mixture was filtered and the carbon black-free culture medium tested and compared to that of macrophages incubated with nontreated culture medium. No significant ($p > 0.05$) differences were found between these comparisons. This result demonstrates that the adsorbates are not being physically released into the tissue culture medium. The observed suppression of phagocytic activity resulted from interaction of the cells with the particle-adsorbate complex and was not caused by free adsorbate in solution.

The effect of adsorbate coverage on phagocytic activity is presented in Figure 5. Acrolein adsorbed onto the

surface of carbon black N339 in concentrations corresponding to 0.5, 1.0, 2.0, and > 2.0 monolayers had a clear dose-dependent effect on suppressing phagocytosis. At concentrations up to 1.0 monolayer, sonification did not remove the adsorbate. At concentration > 2.0 monolayers, sonication was not necessary to remove the adsorbate as the coverage was in excess of 1.0 monolayer and it was physically released from the carbon black into solution in the tissue culture medium. This excess adsorbate suppressed phagocytosis.

Since the majority of these experiments were performed with the N339 incubated with alveolar macrophages for 1 hr at a concentration of 0.04 mg/mL, it was necessary to determine whether the particles were actually ingested by the macrophages. Figure 6 shows that the carbon black particles with this incubation protocol were not ingested but remained associated with the macrophage membrane. At carbon black concentrations of 0.1 and 1.0 mg/mL, it was clearly evident by light microscopy that the agglomerated particles had been internalized.

Since it was experimentally demonstrated that molecules on the surface of carbon blacks have a definite effect on alveolar macrophage Fc-membrane receptor-mediated phagocytosis, actual diesel particulate matter collected from an engine operated under controlled conditions with two different oxidizer formulations was tested. The samples of diesel particulate that were examined in these experiments were collected from an engine operated on a pure hydrocarbon fuel that contained no heteroatoms such as nitrogen or sulfur. The difference between the two samples was due to the presence of nitrogen in the oxidizer. The diesel particulate matter obtained with nitrogen-free oxidizer has been shown to have no direct mutagenic activity by the Ames *Salmonella typhimurium* assay although it could be made to have activity similar to that of the particulate matter obtained with air as the oxidizer (PSU #1) by reaction of the N-free particulate matter with the oxides of nitrogen (11,15). Selected physical and chemical properties of these two diesel particulate samples are shown in Tables 1 and 2 (7,12,16,17). For comparison, these tables also include comparable data for typical carbon blacks, some of which were used in this study, and samples of diesel particulate matter which were collected from an automobile engine operated on commercial diesel fuels (16) (DPM EPA). Figure 7 shows that treatment of the macrophages with PSU #1 had a negligible effect on phagocytosis over the concentrations tested. By comparison, the sample N-free clearly suppressed phagocytosis in a dose-related manner.

Discussion

The rationale for these studies is based on the assumption that alveolar macrophage membrane receptor phagocytotic function may serve as a sensitive method to detect alterations of the bioeffects of carbon black due to the sorption (desorption) of chemicals. The data clearly

FIGURE 6. Transmission electron microscope photomicrograph of a typical alveolar macrophage incubated for 1 hr with 0.04 mg/mL of carbon black N339. The arrow points to the extracellular carbon black particles. $\times 9600$.

Table 1. Elemental analysis of adsorbents.

Particles	% Carbons	% Hydrogen	% Oxygen	% Residue	Empirical formulae
N765	96.35	0.50	1.96	1.19	$C_{669}H_{42}O_{10}$
N339	95.36	0.49	3.61	0.54	$C_{346}H_{21}O_{10}$
N339ox	92.23	0.57	5.39	1.71	$C_{208}H_{13}O_{10}$
N110	96.01	0.26	2.13	1.60	$C_{602}H_{20}O_{10}$
Regal 660	97.56	0.30	1.34	0.76	$C_{971}H_{36}O_{10}$
Mogul L	93.10	0.46	6.14	0.12	$C_{204}H_{12}O_{10}$
Black Pearls					
2000	95.47	0.30	1.76	2.47	$C_{724}H_{27}O_{10}$
DPM PSU #1	95.35	0.65	3.97	0.03	$C_{274}H_{26}O_{10}$
DPM N-free	ND ^a	ND	ND	ND	ND
DPM EPA	89.85	2.66	6.98	0.71	$C_{170}H_{60}O_{10}$

^aND, not determined.

Table 2. Selected physical and chemical properties of adsorbents.

Particles	Surface area, m^2/g	Amount extracted by dichloromethane, mg/g adsorbent	Quality of pyrene in adsorbent, g/g
N765	45	ND ^a	ND
N339	109	ND	ND
N339ox	112	ND	ND
N110	159	ND	ND
Regal 660	95	0.01	3.2×10^{-9}
Mogul L	107	0.01	0.3×10^{-9}
Black Pearls			
2000	1538	ND	ND
DPM PSU #1	104	3.43	310×10^{-6}
DPM N-free	ND	34.5	293×10^{-6}
DPM EPA	70	19.5	ND

^aND, not determined.

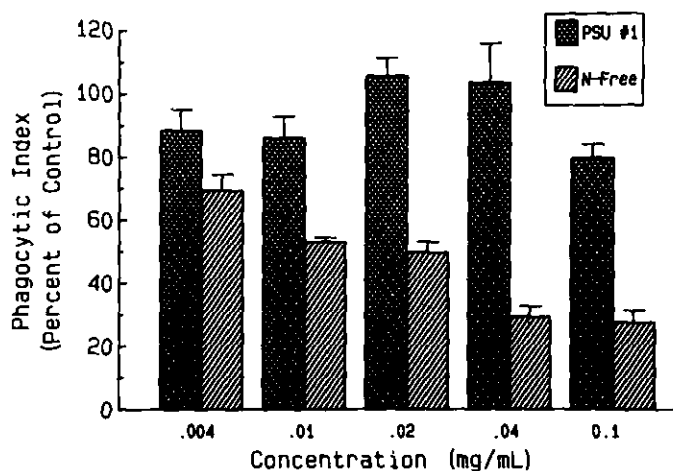


FIGURE 7. Comparison of alveolar macrophage Fc receptor-mediated phagocytosis following 1-hr treatment with diesel engine exhaust samples. Each value represents the mean \pm SE of four determinations.

show that phagocytosis of a secondary particle challenge is affected to varying degrees by both carbon black and adsorbate identity and concentration. Therefore, the assay was judged to be sensitive to the presence and identity of both carbon black and adsorbate.

The carbon blacks used in this study are oil furnace carbon blacks which are produced from the thermal decomposition of oil feed stocks. They have imperfect graphitic structure, are devoid of long-range order, and are considered amorphous (14). The advantage of using these types of particles as opposed to environmental particles is that the carbon blacks are manufactured under well-defined conditions and are, therefore, more reproducible from particle to particle. By changing the carbon black, the parameters contributing to the surface properties may be studied independently. Among the carbon blacks tested, the most dramatic effect on phagocytosis was induced by treatment of the macrophages with Black Pearls 2000 (BP 2000). Physically, BP 2000 is unique because its surface area is approximately 10 times greater than that of the other carbon blacks tested (this black has a greater pore structure) and, in addition, BP 2000 is more spherical than the other carbon blacks. Although the mechanism for the suppression of Fc receptor-mediated phagocytosis induced by prior carbon treatment remains to be elucidated, an explanation may be that the membrane-associated carbon black interacts with or sterically blocks the surface receptors. If this is indeed so, then the enhanced suppression of phagocytosis by BP 2000 may be due to the availability of greater particle surface area for interaction with the Fc receptor. An alternative explanation may be that previous physicochemical studies have shown that this carbon black interacts strongly with all adsorbates studied (7-9). This observation, coupled with the large surface area of BP 2000 could result in increased sorption of molecules onto its surface, thereby altering macrophage function.

Because of the concern that polycyclic aromatic hydrocarbons sorbed on the surfaces of carbon blacks would affect phagocytosis, the endogenous adsorbates were removed and the carbons compared in their native and cleaned states. Removal of the endogenous adsorbates only altered the phagocytosis of macrophages treated with N339ox where the cleaning mitigated the suppression of phagocytosis caused by the native uncleaned particle. An explanation of these results is that N339ox is an oxidized carbon black that is produced by the oxidation of N339 with an oxidizer. Oxidation produces surface functional groups which are basic and have been postulated to be predominantly quinones (7,8). The removal of surface sorbed molecules may result in the exposure of more surface active quinone groups which have increased phagocytic dysfunctional action as compared to the surfaces of the other blacks (7,8).

The adsorbates selected in this study are models for the substituted polyaromatic hydrocarbons found on particulate matter collected from the emissions of the combustion of organic matter. It is reasonable to expect that these model compounds, by virtue of their simplicity as compared to the actual surface sorbed pollutant molecules, would be released more rapidly and thereby produce more phagocytic dysfunction within the time limits of this assay. The results show that macrophage phagocytic dysfunction is clearly concentration and adsorbate dependent and the more hydrophilic adsorbate acrolein produces the greatest effect. The other adsorbates at the higher concentrations do now show the same log dose-response relationship since at these concentrations their aqueous solubilities are exceeded.

The results obtained with the two different samples of diesel particles can be explained on the basis of the amount of sorbed materials that can be extracted with dichloromethane. Although the surface area of diesel particulate matter (DPM) N-free was not determined, it is reasonable to expect that it is comparable to DPM PSU #1. Therefore, since DPM N-free has approximately an order of magnitude more adsorbed molecules than DPM PSU #1, the former particle would be expected to produce increased phagocytic dysfunction on the basis of increased surface coverage. The exact chemical composition of the sorbed molecules on the N-free DPM has not been determined apart from pyrene and 1-nitropyrene, although there is evidence that the N-free DPM contains a greater concentration of oxygen-containing organics that are likely to be more hydrophilic as compared to polyaromatic hydrocarbons (11,15).

The results of this study and related studies (7-9) suggest that work reported by other researchers using doped particles (18) may be in error since it is the coverage of the surface that is a major determinant of release. Studies based on the addition of radiolabeled biologically active compounds to environmental particles do not generally take into account the presence of the endogenous monolayer of combustion-produced adsorbate. The radiolabeled adsorbate, which is sorbed

over a multilayer coverage of material, may be released from the surface more readily than the same adsorbate would be if it were directly sorbed onto the surface active sites. For example, it has already been demonstrated (7) that a monolayer of surface coverage is achieved when diesel particulate matter is only 4% extractable. Clearly, the sample PSU #1 DPM has a coverage that corresponds to a monolayer and it does not cause macrophage dysfunction, whereas the sample N-free DPM has multilayer coverage. It is the facilitated release of adsorbed molecules in concentrations exceeding the monolayer of surface coverage that impairs alveolar macrophage phagocytic function.

In the respiratory tract the alveolar macrophage phagocytic system serves as the primary defense mechanism against inhaled particles that reach the distal lung (19). In the alveoli the macrophages engulf the deposited particles thereby sequestering them from the vulnerable respiratory membrane. The data herein demonstrate that alveolar macrophage phagocytosis is suppressed by exposure to carbon black and adsorbate combinations. This impaired capacity of the macrophages to phagocytize a secondary particle may result in a breach of the defenses in the deep lung. The outcome of this could take several paths. For example, phagocytic dysfunction may lead to an increased susceptibility to pulmonary infections. Alternatively, the increased residence time of particles on the alveolar surface may provide the opportunity of particles to penetrate the interstitium which, in turn, could result in a pathologic outcome. Finally, alveolar macrophages consist of a heterogeneous population (20) functioning not only as phagocytes but also as regulatory cells (21) and antigen presenting cells for the establishment of the specific immune response (22). Whether or not these non-defensive functions of the macrophages are also affected by exposure to carbon black and adsorbate combinations remains to be elucidated.

This research was supported by a grant from the National Institutes of Health R01-ES-03156. The carbon blacks used in this study were generously donated by Cabot Corporation. J.E.F. was supported by a grant from the National Cancer Institute (P30CA06978-26).

REFERENCES

- Boren, H. G. Carbon as a carrier gas mechanism for irritant gases. *Arch. Environ. Health* 8: 127-132 (1964).
- Kilburn, K. H., and McKenzie, W. N. Leukocyte recruitment to airways by aldehyde-carbon combinations that mimic cigarette smoke. *Lab. Invest.* 38: 134-143 (1978).
- Green, G. M., Jakab, G. J., Low, R. B., and Davis, G. S. Defense mechanisms of the respiratory membrane. *Am. Rev. Respir. Dis.* 115: 479-514 (1977).
- Nau, C. A., Neal, J., Stenbridge, V. A., and Cooley R. N. Physiologic effects of carbon black. IV. Inhalation. *Arch. Environ. Health* 4: 415-431 (1962).
- Robertson, J. M., and Ingalls, T. H. A mortality study of carbon black workers in the United States from 1935 to 1974. *Arch. Environ. Health* 35: 181-186 (1980).
- Crosbie, W. A. The respiratory health of carbon black workers. *Arch. Environ. Health* 41: 346-353 (1986).
- Risby, T. H., and Sehnert, S. S. A model for the formation of airborne particulate matter based on the gas-phase adsorption on amorphous carbon blacks. *Environ. Health Perspect.* 77: 131-140 (1988).
- Risby, T. H., Sehnert, S. S., Jiang, L., and Dhingra, B. S. A model for the release of adsorbed molecules from the surfaces of airborne particulate matter based on liquid-phase desorption from amorphous carbon blacks. *Environ. Health Perspect.* 77: 141-149 (1988).
- Sehnert, S. S., and Risby, T. H. Chromatographic modeling of the release of particle-adsorbed molecules into synthetic alveolar surfactant. *Environ. Health Perspect.* 78: 185-195 (1988).
- McLemore, T. L., Warr, G. A., and Martin, R. R. Induction of arylhydrocarbon hydroxylase in human pulmonary alveolar macrophages and peripheral lymphocytes. *Cancer Lett.* 2: 161-168 (1977).
- Herr, J. D., Dukovich, M., Lestz, S. S., Yergey, J. A., Risby, T. H., and Tejada, S. B. The role of nitrogen in the observed direct microbial mutagenic activity for diesel engine combustion in a single-cylinder DI engine. In: *Diesel Engine Combustion, Emissions and Particulates*. Society of Automobile Engineers P-107, Warrendale, PA, 1982, pp. 311-321.
- Yergey, J. A., Risby, T. H., and Lestz, S. S. Chemical characterization of organic adsorbates on diesel particulate matter. *Anal. Chem.* 54: 354-357 (1982).
- Warr, G. A., Jakab, G. J., Hearst, J. E. Alterations in lung macrophage immune receptor(s) activity associated with viral pneumonia. *J. Reticuloendothelial Soc.* 26: 357-366 (1979).
- Riven, D. Carbon Black, In Hutzinger, O., Ed., *The Handbook of Environmental Chemistry, Volume 3/Part D*. Springer-Verlag Berlin Heidelberg, Germany, 1986, pp. 101-158.
- Risby, T. H., and Lestz, S. S. Is the direct mutagenic activity of diesel particulate matter a sampling artifact? *Environ. Sci. Technol.* 17: 621-624 (1983).
- Ross, M. M., Risby, T. H., Steele, W. A., Lestz, S. S., and Yasbin, R. E. Physicochemical properties of diesel particulate matter. *Colloids and Surfaces* 5: 17-31 (1982).
- Risby, T. H., Sehnert, S. S., Jakab, G. J., and Hemenway, D. R. A model to estimate effective doses of adsorbed pollutants on respirable particles and their subsequent release into alveolar surfactant. I. Validation of the model for the adsorption and release of formaldehyde on a respirable carbon black. *Inhal. Toxicol.*, in press.
- Mumford, J. L., Tejada, S. B., Jackson, M., and Lewtas, J. Bioavailability of 1-nitropyrene from model coal fly ash and its uptake by alveolar macrophages. *Environ. Res.* 40: 427-436 (1988).
- Green, G. M., Jakab, G. J., Low, R. B., and Davis, G. S. Defense mechanisms of the respiratory membrane. *Am. Rev. Respir. Dis.* 115: 479-514 (1977).
- Oghiso, Y. Morphologic and functional heterogeneity among rat alveolar macrophage fractions isolated by centrifugation on density gradients. *J. Leukocyte Biol.* 42: 188-196 (1987).
- Scott, W. A., Pawlowski, N. A., Cramer, E. B., and Cohn, Z. A. Secretory functions of the mononuclear phagocyte. *Adv. Exper. Med. Biol.* 183: 17-25 (1985).
- Kaltreider H. B., Caldwell, J. L., and Byrd P. K. The capacity of normal murine alveolar macrophages to function as antigen-presenting cells for the initiation of primary antibody-forming cell responses to sheep erythrocytes in vitro. *Am. Rev. Respir. Dis.* 133: 1097-1104 (1986).